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(54) Title: METHOD, APPARATUS AND KITS FOR SEQUENCING OF NUCLEIC ACIDS USING MULTIPLE DYES

(57) Abstract

An instrument for sequencing oligonucleotides is loaded with the products of four sequencing reaction mixtures. These products are a combination of A, C, G and T reaction products for several sequencing reactions. The products of the different sequencing reactions are labeled with fluorescent tags which are distinguishable one from the other on the basis of their excitation or emission spectra. After separation of the oligonucleotides by electrophoresis, the order of the detected peaks is used to call the base sequence.

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METHOD, APPARATUS AND KITS FOR SEQUENCING OF NUCLEIC ACIDS USING MULTIPLE DYES

DESCRIPTION

BACKGROUND OF THE INVENTION

This application relates to an improved method for sequencing of nucleic acids using multiple fluorescent labels, and to apparatus and kits adapted for use with the method.

Sequencing of nucleic acids using the chain termination method involves the general steps of combining the target nucleic acid polymer to be sequenced with a sequencing primer which hybridizes with the target nucleic acid polymer; extending the sequencing primer in the presence of normal nucleotide (A, C, G, and T) and a chain-terminating nucleotide, such as a dideoxynucleotide, which prevents further extension of the primer once incorporated; and analyzing the product for the length of the extended fragments obtained. Analysis of fragments may be done by electrophoresis, for example on a polyacrylamide gel.

Although this type of analysis was originally performed using radiolabeled fragments which were detected by autoradiography after separation, modern automated DNA sequencers generally are designed for use with sequencing fragments having a fluorescent label. The fluorescently labeled fragments are detected in real time as they migrate past a detector.

US Patent No. 5,171,534 which is incorporated herein by reference describes a variation of this basic sequencing procedure in which four different fluorescent labels are employed, one for each sequencing reaction. The fragments developed in the A, G, C and T sequencing reactions are then recombined and introduced together onto a separation matrix. A system of optical filters is used to individually detect the fluorophores as they pass the detector. This allows the throughput of a sequencing apparatus to be increased by a factor of four, since the four sequencing reaction which were previously run in four separate lanes or capillaries can now be run in one.

It is an object of the present invention to provide a further improvement for use with chain termination sequencing reactions which can increase the throughput of an instrument.

SUMMARY OF THE INVENTION

In order to use nucleic acid sequencing as a diagnostic tool, it will be necessary to determine the sequence of the same DNA region from many samples. The present invention makes it possible to increase the throughput of an instrument being used for this purpose. Thus, a first aspect of the invention provides a method for evaluating the sequence of a target nucleic acid polymer in a plurality of samples. In this method, each sample is first divided into four aliquots which are combined with four sequencing reaction mixtures. Each sequencing reaction mixture contains a polymerase enzyme, a primer for hybridizing with the target nucleic acid, nucleoside feedstocks and a different dideoxynucleoside. This results in the formation of an A-mixture, a G-mixture, a T-mixture and a C-mixture for each sample containing product oligonucleotide fragments of varying lengths. The product oligonucleotide fragments are labeled with spectroscopically-detectable tag, for example a fluorescent tag, and these tags will generally be the same for all four sequencing reactions for a sample. However, the spectroscopically-detectable tags used for each sample are distinguishable one from the other on the basis of their absorption, excitation or emission spectra.

Next, the A-mixtures for each sample are combined to form a combined A mixture, the G-mixtures are combined to form a combined G-mixture and so on for all four mixtures. The combined mixtures are loaded onto a separation matrix at separate loading sites and an electric field is applied to cause the product oligonucleotide fragments to migrate within the separation matrix. The separated product oligonucleotide fragments having the different spectroscopically-detectable tags are detected as they migrate within the separation matrix.

The method of the invention can be used as described above to determine the position of every base in the sequence, or it can be used to determine the position of less than all four bases. For example, the method can be used to determine the position of only the A bases within a sequence for some diagnostic applications.

A further aspect of the present invention is a kit useful for diagnostic sequencing of a selected portion of a gene. One embodiment of such a kit contains a plurality of sequencing primers for the selected portion of the gene, each sequencing primer being identical in its DNA sequence but being labeled with a different spectroscopically-detectable tag.

A further aspect of the invention is an apparatus for performing the method of the invention. Such an apparatus comprises

(a) means for providing excitation energy to a detection site within a separation matrix disposed within the apparatus;

(b) means for detecting light emitted from fluorescently-labeled oligonucleotide fragments located within the detection site;

(c) configuration control means, operatively connected to the means for providing excitation energy and the means for detecting to provide combinations of excitation wavelength and detection wavelength specific for a plurality of different fluorescently-labeled oligonucleotide fragments; and

(d) data processing means, operatively connected to the configuration control means and the means for detecting for receiving a signal from the means for detecting and assigning that signal to a data stream based upon the combination of excitation wavelength and detection wavelength set by the configuration control means.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 A and B shows a schematic representation of the method of the invention;

Figs. 2 A, B, C, and D show excitation and emission spectra for theoretical sets of useful fluorescent tags; and

Fig. 3 shows an apparatus for evaluating the sequence of nucleic acid polymers using the method of the invention.

DETAILED DESCRIPTION OF THE INVENTION

Fig. 1A shows a schematic representation of one embodiment of the method of the invention. The figure depicts the application of the method to two samples for clarity. As will be apparent from the discussion below, however, the method of the invention is not limited to two samples, and is in fact preferably applied for four or more samples, up to a limit imposed only by the number of distinguishable tags which can be identified.

As shown in Fig. 1A, two samples, "sample 1" and "sample 2" are each divided into four aliquots and these aliquots are introduced into sequencing reactions A1, C1, G1, and T1, and A2, C2, G2 and T2. Each sequencing reaction contains the reagents necessary for producing product oligonucleotide fragments of varying lengths indicative of the position of one-base within the target nucleic acid sequence. These reagents include a polymerase

enzyme, for example T7 polymerase, Sequenase™, Thermo Sequenase™, or the Klenow fragment of DNA polymerase; A, C, G and T nucleoside feedstocks; one type of chain terminating dideoxynucleoside; and a sequencing primer.

After the product oligonucleotide fragments are formed in each reaction mixture, the products from reaction mixture A1 are combined with the products from reaction mixture A2 to form a combined mixture 10 which is loaded onto lane 1 of a separation matrix. Likewise, the products from reaction mixture C1 are combined with the products from reaction mixture C2 to form a combined mixture 11 which is loaded onto lane 2 of the separation matrix; the products from reaction mixture G1 are combined with the products from reaction mixture G2 to form a combined mixture 12 which is loaded onto lane 3 of the separation matrix; and the products from reaction mixture T1 are combined with the products from reaction mixture T2 to form a combined mixture 13 which is loaded onto lane 4 of the separation matrix.

The key to the present invention is the use of labels in the reactions A1, C1, G1, and T1 which are distinguishable from the labels used in reactions A2, C2, G2 and T2, respectively. Thus, unlike the method described in US Patent No. 5,171,534 where the labels used for the A, C, G, and T reactions for a sample are distinct, in the present invention the labels used for the four sequencing reactions for any one sample can be, and preferably are, the same. Instead, it is the labels which are used in the several samples which are distinct in the method of the invention.

An alternative embodiment of the invention is illustrated in Fig. 1B. In this case, the operator wants to sequence a plurality of genes (or different exons of the same gene) from one patient sample. The sample 20 is divided into four aliquots. A sequencing reaction mix containing the reagents necessary for producing product oligonucleotide fragments of varying lengths is added to each aliquot. The sequencing mix added to a first aliquot contains all of the reagents for an A termination reaction, plus a plurality of sequencing primers, each one labeled with a distinguishable fluorophore, and each one being specific for a different gene (or different exon of the same gene). The sequencing mix added to a second aliquot contains all of the reagents for a C termination reaction, plus the same plurality of sequencing primers. Sequencing reaction mixes for G and T are made in the same fashion. These sequencing mixture are reacted to produce oligonucleotides fragments, and then loaded onto lanes 21, 22, 23, and 24 of a sequencing gel and separated. Using this technique, any number of genes or

exons in a sample can be simultaneously sequenced up to the limit imposed by the number distinguishable tags which can be identified.

Suitable labels for use in the present invention are fluorescent tags. These can be incorporated into the product oligonucleotide fragments in any way, including the use of fluorescently-tagged primers or fluorescently-tagged chain terminating reagents. Colored dyes detected using absorption spectroscopy can also be employed.

The fluorescent tags selected for use in the present invention must be distinguishable one from another based on their excitation and/or emission spectra. For example, as shown in Fig. 2A, a set of tags could be selected which had overlapping emission spectra (Em1, Em2, Em3 and Em4) but separate and distinguishable excitation spectra (Ex1, Ex2, Ex3, and Ex4). A set of tags could also be selected which had overlapping excitation spectra but separate and distinguishable emission spectra as shown in Fig. 2B. Further, as shown in Fig 2C, a set of tags could be selected in which some of the tags have overlapping excitation spectra (Ex1 and Ex2) but separate and distinguishable emission spectra (Em1 is distinguishable from Em2); while the others have separate and distinguishable excitation spectra (Ex1, Ex3, and Ex4) but overlapping emission spectra (Em1, Em3 and Em4). A further combination of excitation and emission spectra is shown in Fig. 2D.

Examples of sets of suitable tags, together with the wavelength maximum for the excitation and emission spectra are shown in Table 1. Many other fluorophores are available that can be used as labels for DNA sequencing reaction products. Such dyes are available from Applied Biosystems, Inc. (Foster City, CA), Molecular Probes, Inc. (Oregon) and others.

Table 1: Fluorescent Dye's suitable for use with the invention

Fluorescent Dye	Excitation Max (nm)	Emission Max (nm)
Texas Red X	599	617
Carboxy-X-Rhodamine	585	612
CarboxyFluorescein	494	521
CarboxyTetraMethylRhodamine	561	591
Carboxycyanine 5.0	650	667

Fig. 3 shows a basic layout for an apparatus for evaluating the sequence of nucleic acid polymers using the method of the invention. Light from a light source 31, which may be for example a laser, a light emitting diode, a laser diode, an incandescent or polychromatic lamp, or any combination of such sources, is passed through an optical filter 32 if necessary to select an appropriate excitation wavelength which is directed to a detection site 33 in a separation matrix 34. Additional optical components, not shown, may be included as necessary to direct light to the separation matrix. Light emitted by fluorescent tags in the detection site 33 passes through a second optical filter 35 to a detector 36. Again, additional optical components can be included to direct light from the separation matrix 34 to the detector 36. Either or both of the optical filters 32 and 36 may be adjustable under the control of a microprocessor, minicomputer or personal computer 37 to provide various configurations of excitation and emission wavelengths as discussed more fully below. The output from the detector is then transmitted to a data processing system such as a dedicated microprocessor, minicomputer or personal computer 37 for analysis to produce a report on the sequence of the sample being evaluated.

In the case where the properties of the selected tags are of the type shown in Fig. 2A, the optical filter 32 may adjustable, for example by rotating several different filters through the path of the excitation beam, to produce excitation beams corresponding to the different

excitation wavelengths of the tags. An acousto-optic tunable filter of the type employed in U.S. Patent No. 5,556,790, which is incorporated herein by reference, can also be used to separate excitation wavelengths. Optical filter 35 may then be simply a cut-off filter selected to exclude light of the excitation wavelengths from the detector. Information concerning the position of the optical filter 32 as a function of time is transmitted to the data processing system, and used to permit interpretation of the fluorescence data. Thus, when the optical filter 32 is in a position that corresponds to the excitation spectrum of the tag used to label sample 1, the data processing system interprets the emission intensity as data for the sequence of sample 1; when the optical filter 32 is in a position that corresponds to the excitation spectrum of the tag used to label sample 2, the data processing system interprets the emission intensity as data for the sequence of sample 2 and so on for as many different tags are used.

In the case where the properties of the selected tags are of the type shown in Fig. 2B, the optical filter 35 is adjustable, for example by rotating several different filters through the path of the excitation beam, to selectively collect emissions wavelengths corresponding to the different tags. Optical filter 32 may be simply a cut-off or band-pass filter selected to exclude light of the emission wavelengths from the detector. Information concerning the position of the optical filter 35 as a function of time is transmitted to the data processing system, and used to permit interpretation of the fluorescence data. Thus, when the optical filter 35 is in a position that corresponds to the emission spectrum of the tag used to label sample 1, the data processing system interprets the emission intensity as data for the sequence of sample 1; when the optical filter 35 is in a position that corresponds to the emission spectrum of the tag used to label sample 2, the data processing system interprets the emission intensity as data for the sequence of sample 2 and so on for as many different tags as are used.

Finally, in the case where the properties of the selected tags are of the type shown in Fig. 2C, both optical filter 32 and optical 35 are adjustable in synchronization to control the excitation and emission wavelengths being monitored. Information concerning the position of the optical filters 32 and 35 as a function of time is transmitted to the data processing system, and used to permit interpretation of the fluorescence data. Thus, when optical filter 32 is in a position that corresponds to excitation spectrum Ex1 in Fig 2C, and optical filter 35 is in a position that transmits the light of the wavelength of emission spectrum Em1, the data processing system interprets the emission intensity as data for the sequence of the sample

labeled with tag 1. When the optical filter 35 is in a position to transmit light of the wavelength of emission spectrum Em2, on the other hand, the data processing system interprets the emission intensity as data for the sequence of the of sample labeled with tag 2.

When absorption of light rather than emission of light is monitored, the apparatus still has substantially the structures shown in Fig. 3. In this case, however, any optical filters employed before and after the separation matrix are selected to pass light of the same wavelength, corresponding to an absorption of the selected spectroscopically-detectable label.

The light source 31 in the apparatus of the invention may be a single light source which is moved across the gel to irradiate each detection zone individually, for example as described in US Patent No. 5,207,880 which is incorporated herein by reference. The light source 31 may also be a singe light source which is split into multiple beamlets, for example using optical fibers or through the use of a beam splitter such as a spot array generation grating to each of the detection sites as described generally in US Patent Application Serial No. 08/353,932 and PCT Patent Application No. PCT/US95/15951 which are incorporated herein by reference. The light source 31 may also be multiple individual light sources, each of which irradiates a subset of one or more of the detection sites within the separation matrix.

While optical filter 32 described above can be used effectively to provide selection of excitation wavelength, it will be understood that other approaches to providing different excitation wavelengths can be used as well. For example a plurality of lasers of different wavelengths could be used, with the light from each directed in turn to the detection site. For detection of product oligonucleotide fragments in multiple lanes of an electrophoresis gel, one set of lasers might be used (one for each necessary wavelength) with light from each of the lasers being conducted by optical fibers or through the use of a beam splitter such as a spot array generation grating to each of the detection sites as described generally in US Patent Application Serial No. 08/353,932 and PCT Patent Application No. PCT/US95/ 15951. Optical switches, operatively connected to the data processing system, are used to select which of the excitation wavelengths is striking the detection zone at any given time, in the same manner as a rotating optical filter could do. A detector assembly which scans across the lanes of an electrophoresis gel could also be used, for example as described in US Patents Nos. 5,360,523 and 5,100,529, which are incorporated herein by reference, although the time

required for such a device to scan all the lanes of a gel may be a limiting factor in applications with short total migration times.

It will also be appreciated that multiple optical filters mounted on individual detectors could be used in place of the adjustable optical filter 35 and the single detector 36 shown in Fig. 3. Similarly several detectors with adjustable optical filters might also be used.

Other optical components which separate light by wavelength may also be used in place of optical filter 35. Thus, for example, a diffraction grating or prism which spatially separates light of differing wavelength may be employed. In this case, radiation from the different fluorophores will be distributed in space, and can be detected by dedicated detectors such as photodiodes, CCD elements (linear or X-Y). Similarly, distinct optical filters for each wavelength can be employed in combination with a multiplicity of detectors. Optical filters which are transmissive at the emission wavelength of one fluorophore and reflective at the emission wavelength of a second fluorophore can also be used to direct emitted light to separate detectors depending on wavelength.

The detectors used in the apparatus of the invention may be photomultipliers, photodiodes or a CCD. As noted above, the apparatus can be configured with one or more detectors for each detection site. The apparatus can also be configured such that one detector overlaps with several detection sites if the excitation light is directed to the detection sites one at a time. In addition, the apparatus can use a single detector (or a small array of detectors) which is scanned across the gel during detection.

For determining the sequence of a selected region of DNA using the method of the invention, a kit may be formulated. Such a kit comprises, in packaged combination, a plurality of containers, each containing a reagent for the sequencing of the selected region of DNA. The reagent in each container has a reactive portion, which is involved in the sequencing reaction, and a fluorescent label portion. The label portions of the reagents in each container are different and distinguishable one from the other on the basis of the excitation or emission spectra thereof.

In a preferred embodiment, a kit in accordance with the invention comprises a plurality of primers for sequencing the selected region, each of the primers having a different and distinguishable fluorescent label. Thus, the reactive portions of the reagents in this case are the oligonucleotide primer to which the labels are attached. The reactive portions may be

different from one another, but are preferably the same. Such a kit may also include additional reagents for sequencing, including polymerase enzymes, dideoxynucleosides and buffers.

Alternatively, the kit may contain one primer for the selected region and a plurality of containers of chain-terminating nucleotides, each labeled with a different and distinguishable fluorescent label. In this case, the reactive portion of the reagent is the chain terminating nucleoside which can be incorporated in place of a normal nucleoside during the sequencing reaction. The kit may include reagents having just one type of chain-terminating mucleoside, for example ddA with a plurality of distinct fluorescent labels, or it may include reagents having two or more types of chain-terminating nucleotides, each with a plurality of distinct labels. Such a kit may also include additional reagents for sequencing, including polymerase enzymes and buffers, as well as additional chain-terminating nucleotides (single-labeled) for those not provided as part of a multi-label reagent set.

For practicing the method shown in Fig. 1B, a suitable kit in accordance with the invention includes at least one container containing a mixture of a plurality of sequencing primers, one for each gene region to be evaluated. The plurality of sequencing primers each comprise a reactive portion which hybridizes with DNA in the sample and a label portion, the label portions of the reagents being different and distinguishable one from the other. Preferably, the detectable labels are spectroscopically-detectable tags, distinguishable one from the other by their emission or excitation spectra.

CLAIMS

1. A method for evaluating the nucleic acid sequence of a plurality of samples comprising the steps of

(a) obtaining a first aliquot of each sample;

(b) combining the first aliquot of each sample with a sequencing reaction mixture containing a polymerase enzyme, a primer for hybridizing with the sample, nucleoside feedstocks and a first dideoxynucleoside to form a first plurality of mixtures of product oligonucleotide fragments, one for each sample, wherein the product oligonucleotide fragments formed from each first aliquot are labeled with different spectroscopically-detectable tags, said different spectroscopically-detectable tags being distinguishable one from the other on the basis of their excitation or emission spectra;

(c) combining the first plurality of mixtures of oligonucleotide products to form a first combined mixture;

(d) loading the first combined mixture onto a separation matrix at a first loading site;

(e) applying an electric field to cause the oligonucleotide products to migrate within the separation matrix; and

(f) detecting the oligonucleotide products having the different spectroscopically-detectable tags as they migrate within the separation matrix.

2. A method for evaluating the sequence of a target nucleic acid polymer in a plurality of samples comprising the steps of

(a) obtaining four aliquots of each sample;

(b) combining the aliquots of each sample with four sequencing reaction mixtures, each sequencing reaction mixture containing a polymerase enzyme, a primer for hybridizing with the target nucleic acid, nucleoside feedstocks and a different dideoxynucleoside to form an A-mixture, a G-mixture, a T-mixture and a C-mixture for each sample containing product oligonucleotide fragments of varying lengths, wherein the product oligonucleotide fragments are labeled with spectroscopically-detectable tags, and the spectroscopically-detectable tags used for each sample are distinguishable one from the other on the basis of their excitation or emission spectra;

(c) combining the A-mixtures, the G-mixtures, the T-mixtures and the C-mixtures for each sample to form a combined A-mixture, a combined G-mixture, a combined t-mixture and a combined C-mixture;

(d) loading the combined A-mixture, the combined G-mixture, the combined T-mixture and the combined C-mixture onto a separation matrix at separate loading sites;

(e) applying an electric field to cause the product oligonucleotide fragments to migrate within the separation matrix; and

(f) detecting the product oligonucleotide fragments having the different spectroscopically-detectable tags as they migrate within the separation matrix.

3. A method for evaluating the sequence of a plurality of gene regions within a sample comprising the steps of:

(a) combining at least a first aliquot of the sample with a sequencing reaction mixture containing a polymerase enzyme, a plurality of sequencing primer species, one sequencing primer species for each gene region, nucleoside feedstocks and a first dideoxynucleoside to form a first mixture of product oligonucleotide fragments, wherein each of the sequencing primer species is labeled with a different detectable label, said different detectable labels being distinguishable one from the other by a detection system;

(b) separating the first mixture of product oligonucleotide fragments based upon the size of the fragments;

(c) detecting emissions from the separated oligonucleotide fragments for each different detectable label; and

(d) evaluating the sequence of each gene region based upon the oligonucleotide fragments detected.

4. The method according to claim 1 or 3, further comprising the steps

(i) obtaining a second aliquot of each sample;

(ii) combining the second aliquot of each sample with a sequencing reaction mixture containing a polymerase enzyme, a primer for hybridizing with the sample, nucleoside feedstocks and a second dideoxynucleoside different from the first dideoxynucleoside to form

a second plurality of mixtures of product oligonucleotide fragments, one for each sample, wherein the product oligonucleotide fragments formed form each second aliquot are labeled with different spectroscopically-detectable tags, said different spectroscopically-detectable tags being distinguishable one from the other on the basis of their excitation or emission spectra;

(iii) combining the second plurality of mixtures of oligonucleotide products to form a second combined mixture;

(iv) loading the second combined mixture onto a separation matrix at a second loading site, distinct from the first loading site;

(v) applying an electric field to cause the oligonucleotide products to migrate within the separation matrix; and

(vi) detecting the oligonucleotide products having the different spectroscopically-detectable tags as they migrate within the separation matrix.

5. The method according to claim 4, wherein the first and second aliquots are combined with a sequencing reaction concurrently, and wherein the first and second combined mixtures are loaded onto different lanes of the same gel.

6. The method according to claim 5, wherein the same primer and spectroscopically-detectable tag are combined with the first and second aliquots of each sample.

7. The method according to claim 1, wherein first aliquots from at least three samples are combined to form the first combined mixture.

8. The method according to claim 1, wherein first aliquots from more than four samples are combined to form the first combined mixture.

9. The method according to any of claims 1 to 8, wherein the spectroscopically-detectable tag is a fluorescent tag.

10. An apparatus for evaluating the sequence of a nucleic acid polymer by separation of a reaction mixture containing oligonucleotide fragments in a separation matrix disposed within the apparatus comprising

(a) means for providing excitation energy to a detection site within the separation matrix;

(b) means for detecting light emitted from fluorescently-labeled oligonucleotide fragments located within the detection zone;

(c) configuration control means, operatively connected to the means for providing excitation energy and the means for detecting to provide combinations of excitation wavelength and detection wavelength specific for a plurality of different fluorescently-labeled oligonucleotide fragments; and

(d) data processing means, operatively connected to the configuration control means and the means for detecting for receiving a signal from the means for detecting and assigning that signal to a data stream based upon the combination of excitation wavelength and detection wavelength set by the configuration control means.

11. A kit for determining the sequence of a selected region of DNA comprising, in packaged combination, at least of first set of a plurality of containers, each container containing a reagent species for the sequencing of the selected region of DNA, wherein the reagent species in each container comprises a reactive portion and a label portion and wherein the label portions of the reagents are different and distinguishable one from the other.

12. A kit for evaluating the sequence of a plurality of gene regions within a sample comprising, in packaged combination, at least a first container containing a mixture of a plurality of sequencing primers, one for each gene region to be evaluated, wherein the plurality of sequencing primers each comprise a reactive portion which hybridizes with DNA in the sample and a label portion and wherein the label portions of the reagents are different and distinguishable one from the other.

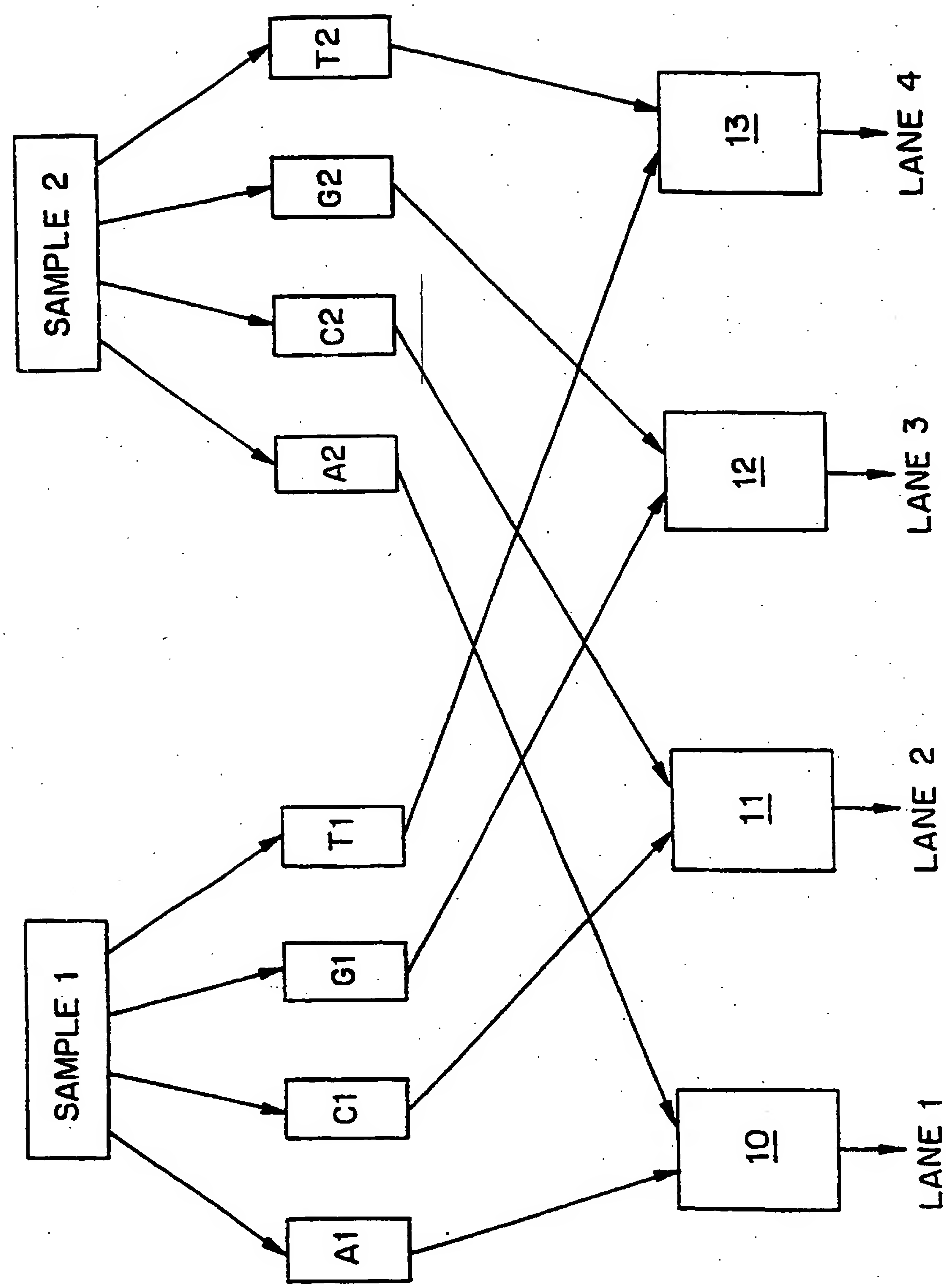
13. The kit according to claim 11 or 12, wherein the detectable labels are spectroscopically-detectable tags, distinguishable on the basis of the excitation or emission spectra thereof.

14. The kit according to any of claims 11 through 13, wherein the reactive portion of each reagent species in the first set of containers is an oligonucleotide primer for use in sequencing the selected region of DNA.

15. The kit according to claim 14, wherein the reactive portions of each reagent species in the first set of containers are the same as one another.

16. The kit according to claim 11, wherein the reactive portion of each reagent species in the first set of containers is a dideoxynucleoside and wherein the reactive portions of each reagent species in the first set of containers are the same as one another.

17. The kit according to any of claims 11 or 12 through 16, further comprising a second set of containers, each containing a reagent species for the sequencing of the selected region of DNA, wherein the reagent species in each container of the second set comprises a dideoxynucleoside reactive portion different from the reactive portion of the reagent species in the first set of containers, and a fluorescent label portion and wherein the label portions of the reagents in the second set of containers are different and distinguishable one from the other on the basis of the excitation or emission spectra thereof.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T2
G2
C2
A2
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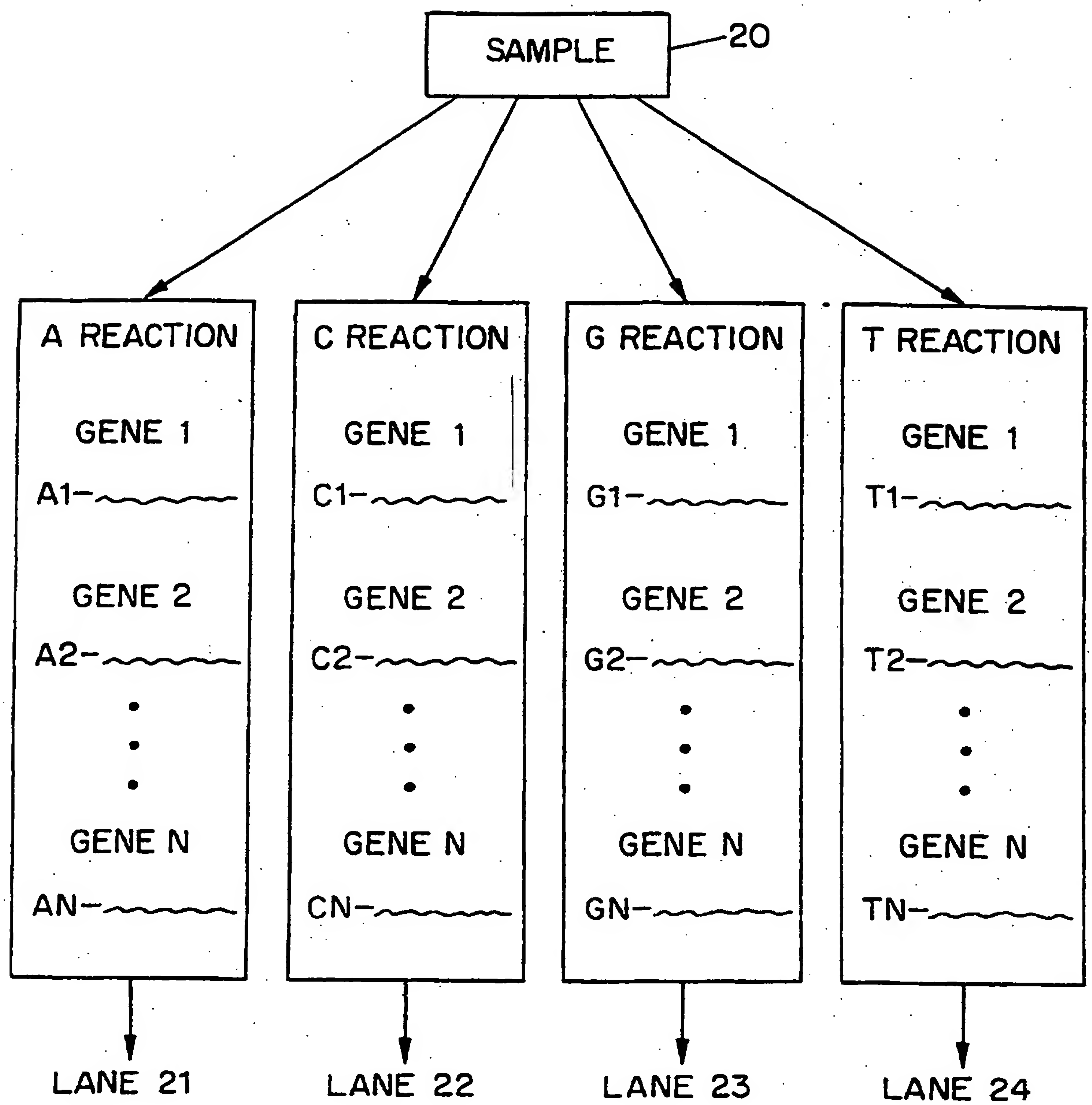
LANE 3
LANE 2
LANE 1

FIG. 1A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GENE 1
A1–
GENE 2
A2–
GENE N
AN–
C REACTION
GENE 1
C1–
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GENE N
CN–
G REACTION
GENE 1
G1–
GENE 2
G2–
GENE N
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GENE 1
T1–
GENE 2
T2–
GENE N
TN–
LANE 21
LANE 22
LANE 23
LANE 24

FIG. 1B

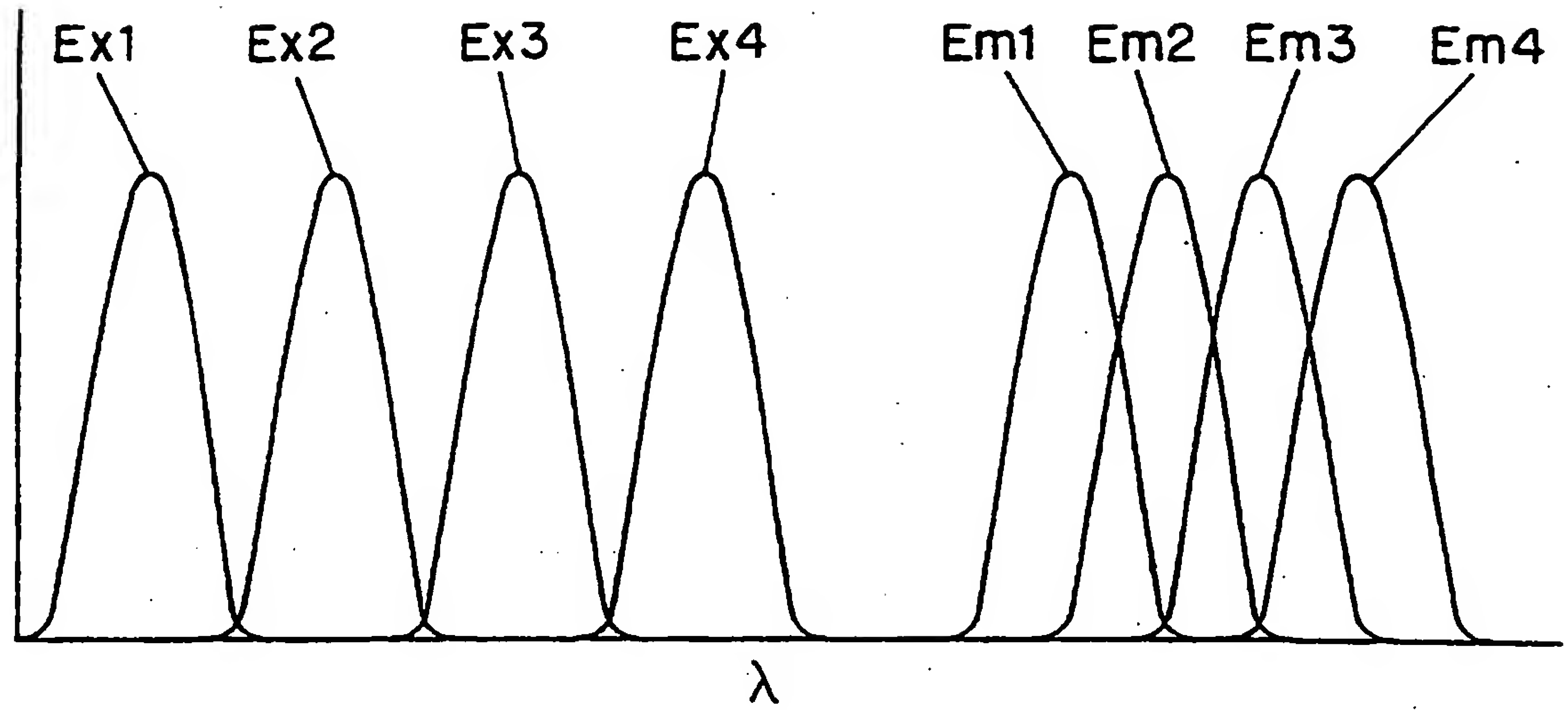

FIG. 2A

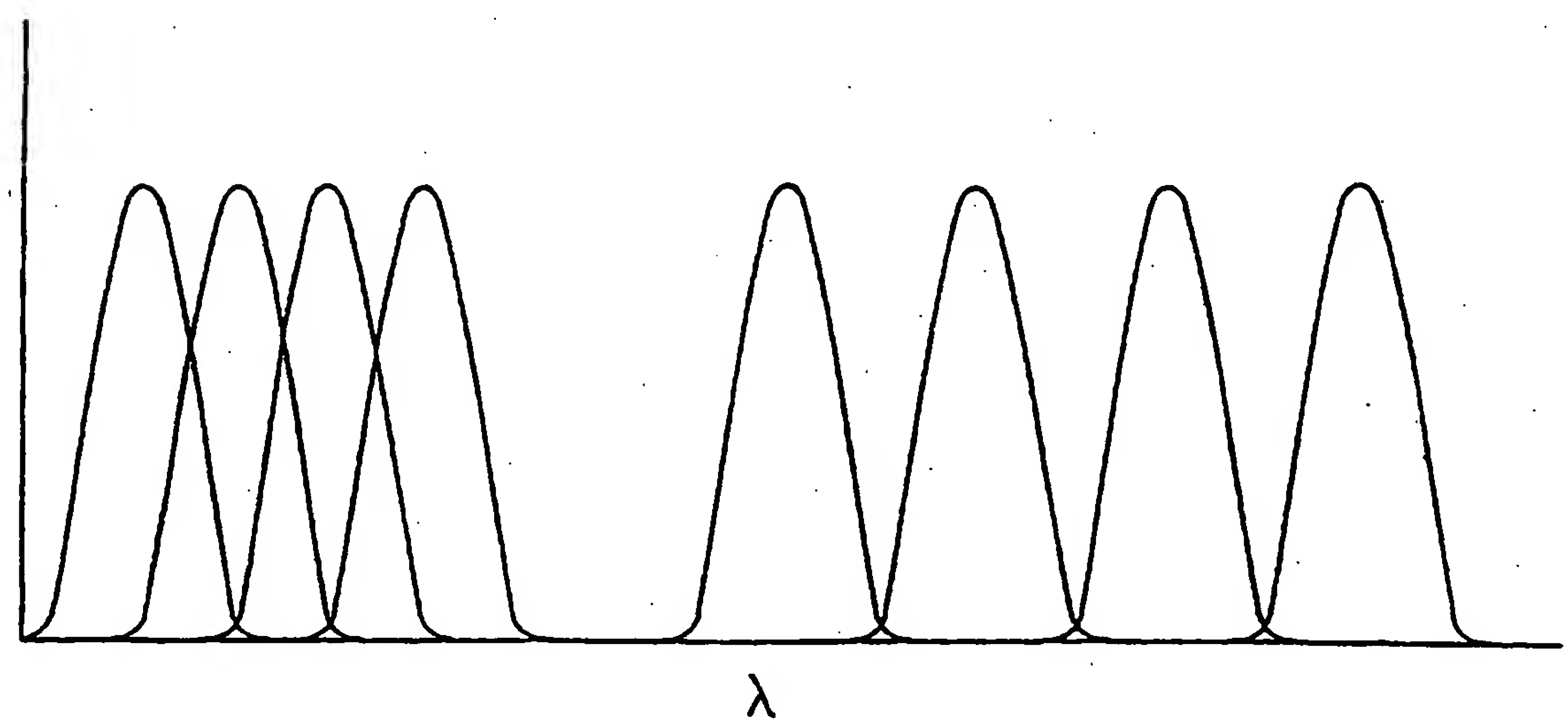

FIG. 2B

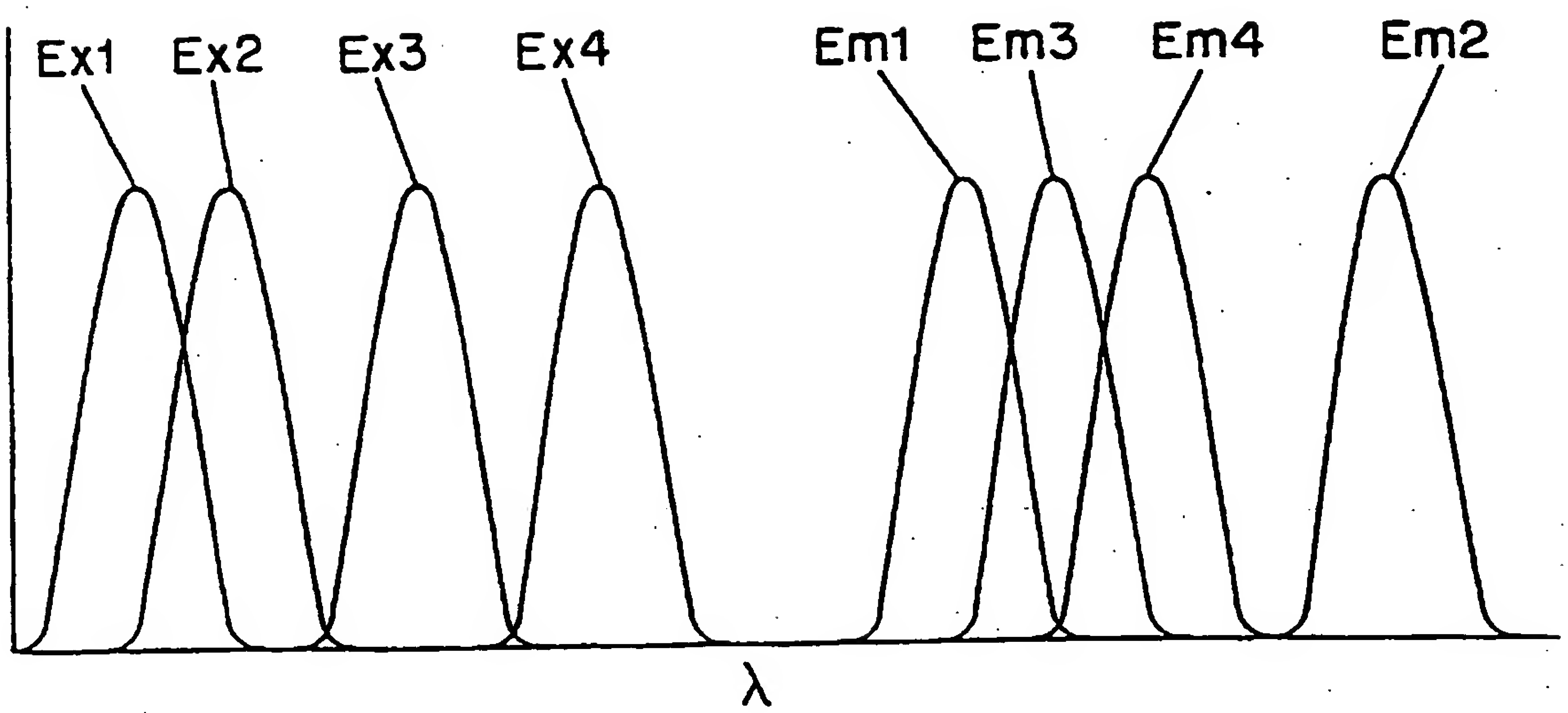
Ex1
Ex2
Ex3
Ex4
Em1
Em3
Em4
Em2
λ

FIG. 2C

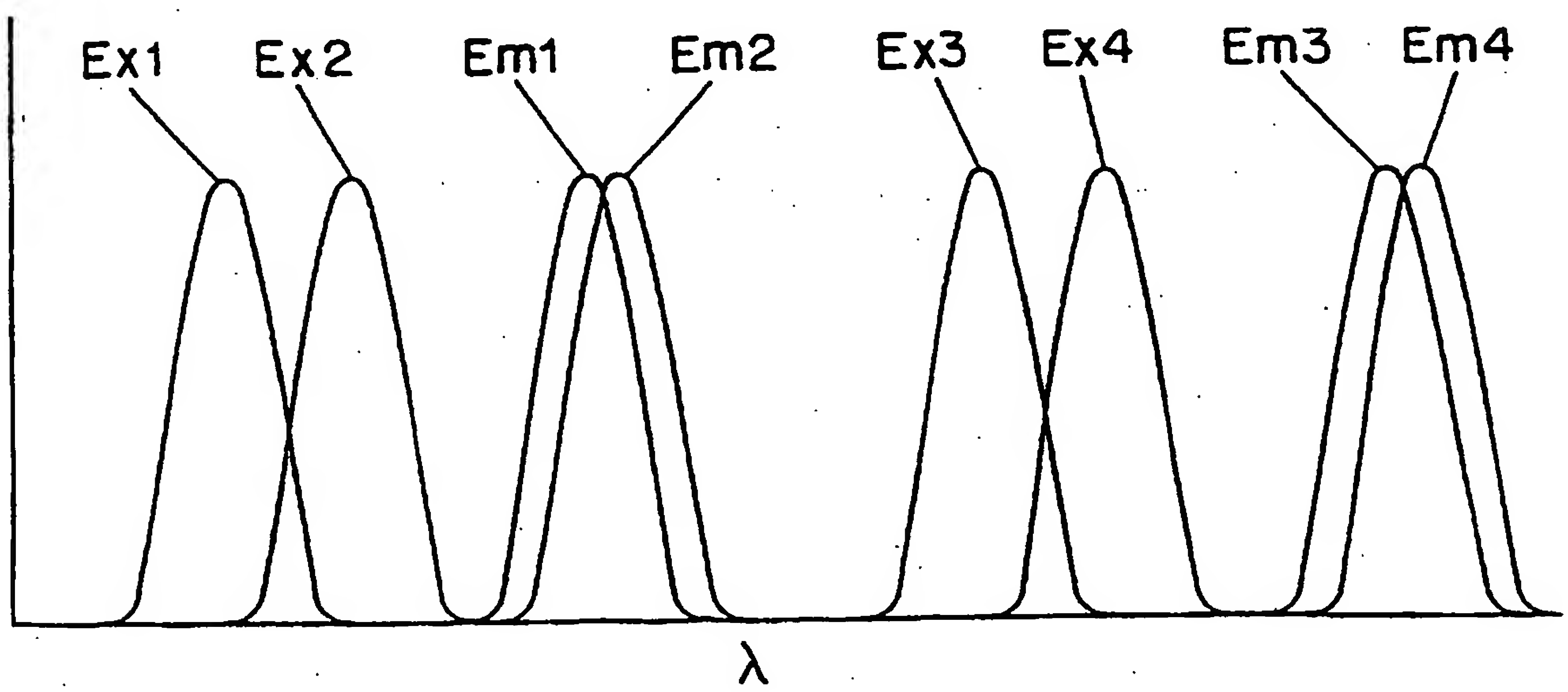
Ex1
Ex2
Em1
Em2
Ex3
Ex4
Em3
Em4
λ

FIG. 2D

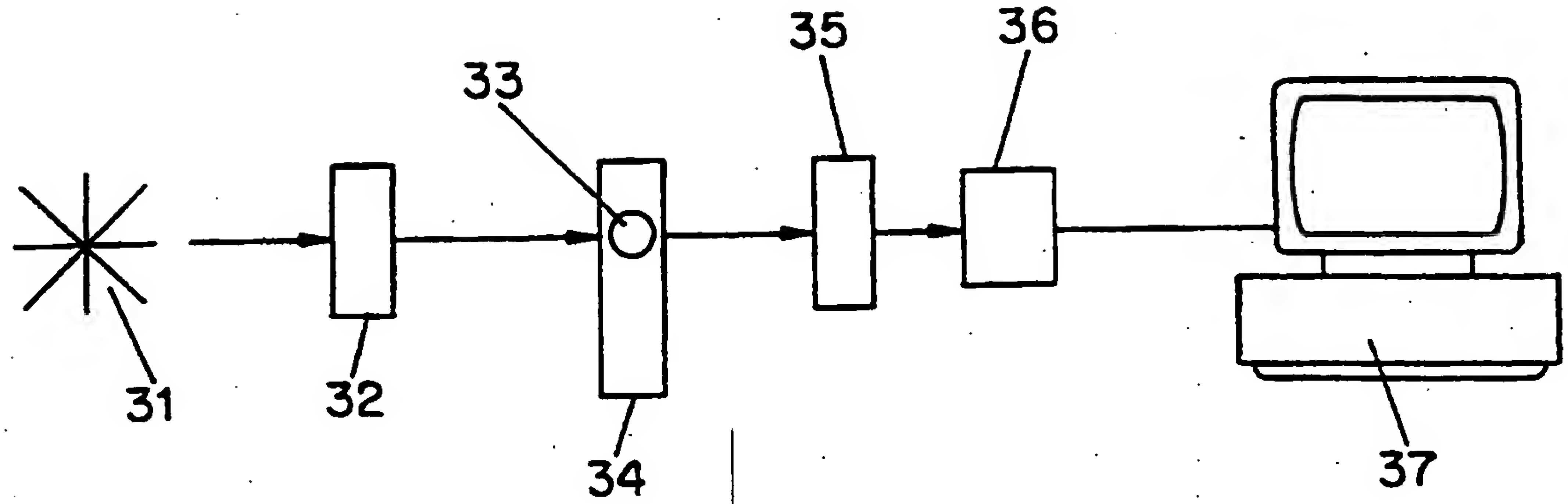

FIG. 3

INTERNATIONAL SEARCH REPORT

Inten nal Application No
PCT/CA 97/00251

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 G01N27/447

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 307 148 A (KAMBARA HIDEKI ET AL) 26 April 1994 see the whole document ---	1-17
X	ANALYTICAL BIOCHEMISTRY, vol. 224, no. 1, 1 January 1995, pages 117-121, XP000486746 WIEMANN S ET AL: "SIMULTANEOUS ON-LINE DNA SEQUENCING ON BOTH STRANDS WITH TWO FLUORESCENT DYES" see the whole document --- -/--	1-17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
20 August 1997	29.08.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hagenmaier, S

Intern   nal Application No
PCT/CA 97/00251

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIO/TECHNOLOGY, vol. 9, no. 7, July 1991, pages 648-651, XP000601481 KAMBARA H ET AL: "REAL TIME AUTOMATED SIMULTANEOUS DOUBLE-STRANDED DNA SEQUENCING USING TWO-COLOR FLUORPHORE LABELING" see the whole document ---	1-17
X	BIOTECHNIQUES, vol. 15, no. 3, 1993, pages 480-485, XP000389937 OLESEN ET AL.: "Chemiluminescent DNA sequencing with multiplex labeling" see the whole document -----	1-17

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern nal Application No
PCT/CA 97/00251

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5307148 A	26-04-94	JP 3293557 A	25-12-91